Research Protocol

1. Summary

Auditory-verbal hallucinations are experienced by most patients with schizophrenia and often lead to distress, disability, and an increased risk of violence or suicide. Auditory-verbal hallucinations are typically treated with antipsychotic medications, but approximately 25% of patients do not experience satisfactory effects from pharmacological treatment. It has been suggested that these hallucinations arise because brain areas associated with language functions are overactive, while brain areas responsible for controlling internal voices are, at the same time, not active enough. Transcranial direct current stimulation (tDCS) is a non-invasive technique that can temporarily increase or decrease activity in specific brain regions. The proposed research project will investigate whether activating brain areas responsible for control and inhibiting brain areas for language using tDCS can influence auditory-verbal hallucinations in patients with schizophrenia. To achieve this, two experiments are proposed:

In the first experiment (Main Experiment), half of the patients will receive real tDCS (2 × 20-minute sessions per day for five consecutive days), while the other half will receive sham tDCS (control). Auditory-verbal hallucinations will be assessed before (baseline), immediately after (acute), and three months after tDCS (follow-up). Potential changes in neuronal activity and neural systems will be examined using magnetic resonance imaging (MRI) of the brain—also at baseline, acute, and follow-up time points. Finally, the effects of tDCS on general functioning and cognitive abilities will be assessed using a neurocognitive test battery. In the second experiment (Online Experiment), patients with frequent auditory-verbal hallucinations will indicate when they are experiencing hallucinations while simultaneously receiving tDCS, and their neuronal activity will be recorded using MRI. This allows for real-time investigation of the underlying neuronal mechanisms behind the effects of tDCS. Overall, the aim of the proposed project is to investigate the potential of tDCS in reducing auditory-verbal hallucinations in schizophrenia. In a medium- to long-term perspective, this could pave the way for tDCS as a complementary treatment for medication-resistant auditory-verbal hallucinations.

2. Theoretical background

Schizophrenia is a mental disorder with severe consequences for both patients and society. Auditory-verbal hallucinations are a core symptom of schizophrenia, affecting approximately 70% of patients (Hugdahl, 2008). Patients typically report that the voices they hear contain malicious or rude content, such as "you are stupid." Sometimes, the voices may command patients to commit violent acts against others or themselves (Cheung et al., 1997). Naturally, this leads to distress and severe impairments in behavioral control and social functioning. Auditory-verbal hallucinations are usually treated with medication, but up to 25% of schizophrenia patients do not respond satisfactorily to pharmacological treatment (for a review, see Shergill, Murray & McGuire, 1998).

Based on previous research, Hugdahl (2009) developed a model postulating that auditory-verbal hallucinations in schizophrenia arise due to abnormal activations in two brain regions. First, it is assumed that left-hemispheric language areas are overactive in schizophrenia patients compared to healthy individuals. Such hyperactivity generates acoustic experiences perceived as voices. This perspective is supported by neuroimaging findings showing that the left peri-Sylvian language region is activated during auditory hallucinations (Lennox et al., 2000) and is generally more active in the

absence of auditory stimuli (Kompus et al., 2011). Second, frontal regions, which are critical for attention and cognitive control, are thought to be less active in schizophrenia patients compared to healthy individuals. As a result, schizophrenia patients are unable to control the voices they hear. Reduced cognitive control is a typical finding, and several neuroimaging studies report "hypofrontality" in schizophrenia patients, meaning hypoactivation of the prefrontal cortex (see Ragland et al., 2007).

If the hypertemporal-hypofrontality model is correct, one would expect that increased neuronal activity in prefrontal regions and decreased neuronal activity in the peri-Sylvian language area would reduce auditory-verbal hallucinations. Non-invasive brain stimulation techniques such as Transcranial Magnetic Stimulation (TMS) and the more recent Transcranial Direct Current Stimulation (tDCS) allow for transient modulation of cortical excitability. In TMS, rapidly changing magnetic fields are generated around a coil placed on the scalp. This induces a weak, short-lived current in the underlying brain tissue, triggering action potentials. Depending on several factors, including stimulation frequency and duration, TMS can have either excitatory or inhibitory effects on the stimulated brain region (Cowey, 2005). In tDCS, two electrodes (anode and cathode) delivering a constant electric current are placed on the scalp. This modulates the overall excitability of neurons, leading to "a tonic de- or hyperpolarization of the membrane's resting potential." Typically, neuronal activation under the anode is facilitated, while neuronal activation under the cathode is inhibited (Nitsche et al., 2008; Stagg & Nitsche, 2011).

As early as 1999, Hoffman and colleagues applied low-frequency, inhibitory TMS to the left temporoparietal cortex (just behind the peri-Sylvian language area) and found a reduction in auditory-verbal hallucinations. Although the initial findings were promising (Hoffman et al., 2000; Poulet et al., 2005), more recent studies with large sample sizes and carefully controlled designs have not found support for the effectiveness of TMS (Slotema et al., 2011; see also de Jesus et al., 2011). There have also been attempts to stimulate the left prefrontal regions using excitatory TMS protocols. If anything, this procedure has shown potential to improve negative symptoms such as anhedonia, lack of speech, or emotional flattening, but findings have been mixed, and there is no evidence of improvements in auditory-verbal hallucinations (Prikryl et al., 2007). Simultaneous stimulation of (left) frontal and temporal regions has not been attempted with TMS. This would be impractical, as two coils would be required, which could interfere with each other, and dual stimulation might induce epileptic seizures. In contrast to TMS, tDCS does not have these issues. The anode (excitatory effect) and cathode (inhibitory effect) can easily be placed over the temporal and frontal regions. Additionally, since tDCS does not trigger action potentials, dual stimulation of two brain areas with tDCS remains well within safety parameters (Nitsche et al., 2008). Despite these clear advantages of tDCS over TMS, only one study so far has used tDCS to treat auditory-verbal hallucinations. Inhibitory temporal and excitatory frontal stimulation led to a significant reduction in auditory-verbal hallucinations in medication-resistant schizophrenia patients (Brunelin et al., 2012).

Although this finding is promising, more studies are needed to evaluate the effects of tDCS on auditory-verbal hallucinations. It should also be noted that Brunelin et al. did not use neuronavigation systems to determine the exact location of the dorsolateral prefrontal cortex and the peri-Sylvian language area in each individual. Therefore, it is unclear whether the electrodes were placed correctly. Additionally, no neuroimaging procedures were conducted before, during, or after tDCS. A direct test of whether neuronal activity in frontal and temporal areas was stimulated or inhibited by tDCS is still lacking.

Moving from neuronal activity to neurotransmitters, in healthy individuals, anodal tDCS has been shown to reduce γ-aminobutyric acid (GABA) concentrations in the stimulated brain region (Stagg et

al., 2009). Since GABA is the primary inhibitory neurotransmitter, a reduction in GABA is consistent with the excitatory effects of anodal tDCS (Stagg et al., 2009). Cathodal tDCS leads to a reduction in glutamate concentrations, and since glutamate is the main excitatory neurotransmitter, this could explain the inhibitory effects of cathodal tDCS. Schizophrenia has been proposed to be characterized by a hypofunctional glutamate system and an imbalance between excitatory and inhibitory neurotransmitters (Kehrer et al., 2008). Thus, one could hypothesize that if tDCS over the prefrontal cortex and the peri-Sylvian language area successfully reduces auditory-verbal hallucinations, this would be reflected in increased glutamate levels in the left prefrontal cortex and increased GABA levels in the left peri-Sylvian region. So far, no study has investigated neurotransmitter levels, and in general, the underlying neuronal mechanisms of possible tDCS effects in schizophrenia patients remain unknown.

3. Methods

Two experiments are proposed to investigate (a) the potential of tDCS to affect auditory-verbal hallucinations and (b) its underlying neuronal mechanisms.

3.1 Recruitment of participants

Schizophrenia patients with auditory-verbal hallucinations for both experiments will be recruited from Haukeland University Hospital, Division of Mental Health Care, through the Bergen Psychosis Project 2 (BP2). Patient recruitment will be conducted by staff from the Research Department in close collaboration with clinical personnel from the psychiatric clinics. For the Main Experiment, patients must be 18 years or older and must have a psychotic disorder within the schizophrenia spectrum (schizophreniform disorder, schizophrenia, schizoaffective disorder) with auditory-verbal hallucinations. For the Online Experiment, patients must meet the inclusion criteria for the Main Experiment and additionally experience auditory-verbal hallucinations frequently enough to be observed within a 20-minute window—the time required to complete a combined tDCS/fMRI sequence. Patients participating in the Online Experiment will be asked whether they would also like to participate in the Main Experiment to maximize the number of participants in both studies.

To confirm the schizophrenia diagnosis and the presence of auditory-verbal hallucinations, patients will undergo a series of clinical assessments. These assessments will be conducted within the already approved BP2 project (2010/3387-6/REK Vest). Patients who meet the inclusion criteria will be invited to participate in the proposed project. Depending on which experiment they wish to take part in, they will be able to sign an informed consent form for the Main Experiment, the Online Experiment, or both. The informed consent form also specifies that the principal investigator of the proposed research project will have access to relevant data for the respective patient collected within the framework of the BP2 project. This is necessary to ensure that the principal investigator is fully informed of any potential health and safety risks.

3.2 Main experiment

3.2.1 tDCS procedure

The patients will be assigned to one of two conditions: real tDCS and sham tDCS. In the real tDCS condition, patients will receive 2 × 20 minutes of tDCS per day for five consecutive days. There will be

at least a three-hour break between the two daily tDCS sessions, and the electrical current for tDCS will be set at 2 mA. The stimulation follows the protocol of Brunelin et al. (2012) and adheres to tDCS safety parameters (Nitsche et al., 2008). The anode will be placed over the left prefrontal cortex, and the cathode over the left peri-Sylvian area. Standard-sized electrodes (35 cm^2; 7 cm × 5 cm) will be inserted into sponges soaked in saline solution to improve electrical conductivity. The stimulation will be administered by qualified personnel from the clinical department. In the sham condition, patients will undergo the exact same tDCS procedure as in the real tDCS condition. However, unbeknownst to both the patients and the staff administering the tDCS (double-blind design), the stimulator will be turned off after a few seconds of stimulation. This ensures that neuronal activity in the "stimulated" brain area remains unchanged while the participants still experience the initial tingling sensation of tDCS, giving them the impression that they are receiving real stimulation. A series of assessments will be conducted at three time points: before tDCS (baseline), immediately after tDCS (acute), and three months after the tDCS procedure (follow-up) to determine its effects and investigate the underlying neuronal mechanisms. Patients in the real and sham conditions will be matched for gender and general intelligence. An overview of the experimental design is provided in Figure 1 below.

3.2.2 Auditory-verbal hallucinations

At study onset, immediately post-treatment, and during follow-up, auditory hallucinations will be assessed using the Positive and Negative Syndrome Scale (PANSS) and the Auditory Hallucination Rating Scale (AHRS). PANSS data will be collected as part of the BP2 project. Additionally, patients will be provided with an iPhone/iPod equipped with an application that allows them to rate their hallucinations along five dimensions on a continuum:

- **Localization** (internal – external)
- **Content** (negative – positive)
- **Control** (none – full)
- **Frequency** (low – high)
- **Intensity** (low – high)

This application was developed by the fMRI group in Bergen and offers the advantage of enabling patients to provide quick, simple, and unfiltered self-reports. Patients will rate their hallucinations daily from the first day of tDCS stimulation until the follow-up assessment. The data will be stored anonymously on the iPhone/iPod.

Figure 1. Experimental design

	Pre-study assessment	**Online tDCS Experiment** (In addition for patients with frequent hallucinations)	**Main Experiment** (all patients)				
			Day 1 (baseline)	Days 2 to 6 (tDCS)	Day 7 (acute tDCS effect)		3 months (follow-up)
Clinical examination	SCID-1, AUDIT, DUDIT, demographics, tDCS checklist						
Hallucination assessment	PANSS	Self-ratings	PANSS, AHRS, i-pod hallucination app	i-pod hallucination app	PANSS, AHRS, i-pod hallucination app	i-pod hallucination app	PANSS, AHRS, i-pod hallucination app
Neuroimaging		Two sessions (real + sham) of fMRI compatible tDCS while reporting the presence of hallucinations	Resting state fMRI, dichotic listening fMRI, spectroscopy, DTI, structural MRI		Resting state fMRI, dichotic listening fMRI, spectroscopy, DTI, structural MRI		Resting state fMRI, dichotic listening fMRI, spectroscopy, DTI, structural MRI
Neurocognitive functioning	General & premorbid intelligence		Global functioning, verbal memory, working memory, visuo-motor speed and attention		Global functioning, verbal memory, working memory, visuomotor speed and attention		Global functioning, verbal memory, working memory, visuomotor speed and attention

3.2.3 Neuroimaging

Brain imaging will be conducted using a 3.0 T GE Signa MR scanner at Haukeland University Hospital in Bergen. Before entering the scanner, radiographers will check whether patients have any MR risk factors. Additionally, patients will complete a tDCS checklist to ensure their safety during the combined MR/tDCS recording. Patients will undergo five MR sequences at three time points (baseline, acute, follow-up):

1. **High-resolution anatomical scan** – This will be performed to investigate changes in white/gray matter as a result of tDCS. The sequence will take approximately 10 minutes.
2. **Magnetic Resonance Spectroscopy (MRS)** – This sequence will examine changes in GABA and glutamate concentrations. Based on axial T2-weighted MR images, a voxel (3 x 3 x 3 cm) will be placed in the left peri-Sylvian area and the left prefrontal cortex. GABA and glutamate concentrations will be identified using MEGA-PRESS, a sequence capable of detecting both neurotransmitters (Henry et al., 2011). It is hypothesized that tDCS will reduce GABA concentrations in the prefrontal regions (indicating an excitatory effect) and reduce glutamate concentrations in the temporal regions (indicating an inhibitory effect). The required scanning time per voxel is approximately 8–10 minutes.
3. **Diffusion Tensor Imaging (DTI)** – This sequence will be performed to map white matter and will last approximately 8 minutes.
4. **Resting-State functional MRI (fMRI)** – This sequence will assess whether tDCS increases neuronal activity in hypoactive prefrontal regions and inhibits neuronal activity in hyperactive temporal regions. The sequence duration is about 5 minutes.
5. **Task-based fMRI using the Bergen Dichotic Listening Task** – This is one of the most widely used dichotic listening tasks and has been shown to assess frontal and temporal functional integrity in numerous studies (for a review, see Hugdahl, 1995). The fMRI version of the test consists of six syllables ("ba," "da," "ga," "pa," "ta," "ka"), with two different syllables simultaneously presented to the left and right ear (for more information on the fMRI version, see Hirnstein et al., 2013). 30 dichotic syllable pairs (excluding six homonym pairs such as "ba"
   – "ba") are presented in blocks of ten. In the first three blocks, participants report the

syllable they hear most clearly (free attention condition). In the remaining six blocks, participants report the syllable from either the left or right ear (forced attention condition). The free attention condition measures language asymmetry, while the forced attention condition assesses cognitive control (Hugdahl et al., 2009). Schizophrenia patients typically show a reduced right-ear advantage in the free attention condition (i.e., fewer correct reports from the right ear) and impaired ability to report syllables from the focused ear in the forced condition (Hugdahl, 1995). Our hypothesis is that tDCS will increase right-ear advantage and improve correct reports from the focused ear.

The total duration of the fMRI version of the Bergen Dichotic Listening Test is approximately 18 minutes, bringing the overall scanning time to just over one hour. Before MR scanning, a standard hearing test will be conducted.

3.2.4 General cognitive function

General intelligence can positively influence tDCS outcomes. Therefore, the two groups will be matched for general intelligence and premorbid cognitive abilities, assessed using the Wechsler Abbreviated Scale of Intelligence (WASI) and the National Adult Reading Test (NART), respectively. WASI and NART will only be conducted at baseline. Furthermore, patients will be examined using psychometric tests and a (neuro-)cognitive test battery to investigate whether tDCS has beneficial effects on general functioning and specific cognitive abilities. The test battery includes:

- Global Assessment of Functioning Scale (GAF; separate version)
- Clinical Global Impression Scale – Severity of Illness (CGI-S) (both measure general functioning)
- California Verbal Learning Test II (verbal learning and memory)
- Trail Making Test A & B
- Grooved Pegboard Test (both assess visuomotor speed and attention)
- Block Design (subtest from Wechsler Adult Intelligence Scale) (spatial working memory)
- Letter-Number Span Test (verbal working memory)

All these tests are conducted as part of the already approved BP2 project.

3.3 Online experiment

Patients will lie relaxed in the MRI scanner and press a button whenever they experience auditory hallucinations. This paradigm is well established (e.g., Raij et al., 2009; Dierks et al., 1999) and allows for the determination of both the frequency and duration of hallucinations. This enables the identification of the presence and duration of auditory hallucinations. At the same time, patients will receive either real or sham tDCS for 20 minutes. To achieve this, a special MRI-compatible tDCS device (DC Stimulator MR, manufactured by NeuroConn GmbH, Ilmenau, Germany) with specialized rubber electrodes and cables will be used. Immediately after the 20-minute combined tDCS/fMRI sequence, patients will complete elements of the auditory hallucination program (app) on an iPhone/iPod to specify the content of their hallucinations.

Each patient will undergo the combined tDCS/fMRI sequence twice. Those who received real tDCS in the first condition will receive sham tDCS in the second condition and vice versa. This paired design increases statistical power, and the entire Online Experiment will take approximately 1.5 hours in total.

Our hypothesis is that tDCS will increase neuronal activity in the prefrontal cortex and decrease activity in the temporal language areas, thereby reducing the occurrence and duration of auditory

hallucinations.

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